JC14 Rec'd PCT/PTO 20 JUL 2001

FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 33796

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 09/889802

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/00244	January 29, 2000	January 30, 1999

TITLE OF INVENTION: METHOD AND MEDICAMENT FOR INHIBITING THE EXPRESSION OF A DEFINED GENE

APPLICANT(S) FOR DO/EO/US: Roland Kreutzer and Stephan Limmer

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [X] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article ~~19~~ 34 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [X] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [X] An English language translation of the amendments to the claims under PCT Article ~~19~~ 34 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98; Form PTO-1449.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [X] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information: NOTE: APPLICANT CLAIMS ENTITLEMENT TO SMALL ENTITY STATUS. An "Express Mail" Mailing Label Certificate is attached to this Form PTO-1390 for Express Mail Label EL707689323US.

JC17 Rec'd PCT/PTO 20 JUL 2001

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/889802	PCT/DE00/00244	33796

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

	CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	125 - 20 =	105	x **$18.00**	$ 1,890.00
Independent claims	5 - 3 =	2	x **$80.00**	$ 160.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	n/a		+ **$270.00**	$ 0.00
TOTAL OF ABOVE CALCULATIONS =				$ 3,040.00
[XX] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$ 1,520.00
SUBTOTAL =				$ 1,520.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ 0.00
TOTAL NATIONAL FEE =				$ 1,520.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0.00
TOTAL FEES ENCLOSED =				$ 1,520.00
			Amount to be refunded:	$
			charged:	$

a. [XX] A check in the amount of $ 1,520.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0820 . A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
John P. Murtaugh
Pearne & Gordon LLP
526 Superior Avenue East, Suite 1200
Cleveland, Ohio 44114-1484

John P. Murtaugh
SIGNATURE

John P. Murtaugh
NAME

34226
REGISTRATION NUMBER

"EXPRESS MAIL" MAILING LABEL CERTIFICATE

Re: U.S. PCT-based Patent Application for
"Method and Medicament for Inhibiting the Expression of a Defined Gene"
International Application No.: PCT/DE00/00244
International Filing Date: January 29, 2000
Attorney's Docket No.: 33796

"Express Mail" mailing label number EL707689323US

Date of Deposit July 20, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Commissioner for Patents, ATTN: BOX PCT, Washington, D.C. 20231

Linda Ibbett

Printed Name of Person Mailing Paper or Fee

Linda Ibbett

Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Roland Kreutzer, et al.

Title: METHOD AND MEDICAMENT FOR INHIBITING THE EXPRESSION OF A DEFINED GENE

International Application No.: PCT/DE00/00244

International Filing Date: January 29, 2000

Docket No.: 33796

PRELIMINARY AMENDMENT

Commissioner for Patents
ATTN: BOX PCT
Washington, DC 20231

Sir:

Please amend the application before its examination as follows. It is noted that during the International Phase new claims 1-125 were filed on April 4, 2001, in accordance with PCT Article 34. These claims 1-125 are the claims which are presently pending in this application. These claims 1-125 are now being amended.

IN THE CLAIMS:

Page 1, line 1 of the Amended Sheets, delete the headings "International Patent Application No. PCT/DE00-00244 of Dr Roland Kreutzer and Dr. Stefan Limmer" and "New Patent Claims" and insert therefore starting at the left hand margin --WHAT IS CLAIMED IS:--.

Please amend claims 3-7, 10-31, 34-38, 41-61, 65-80, 83-87, 90-110, 112-113, 116-118, and 121-125 to read as follows:

3. (Amended) Method according to claim 1, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

4. (Amended) Method according to claim 1, where the target gene is expressed in eukaryotic cells.

5. (Amended) Method according to claim 1, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

6. (Amended) Method according to claim 1, where the target gene is expressed in pathogenic organisms, preferably in plasmodia.

7. (Amended) Method according to claim 1, where the target gene is part of a virus or viroid.

10. (Amended) Method according to claim 1, where segments of the dsRNA are in double-stranded form.

11. (Amended) Method according to claim 1, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

12. (Amended) Method according to claim 1, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

13. (Amended) Method according to claim 1, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

14. (Amended) Method according to claim 1, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

15. (Amended) Method according to claim 1, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being

poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

16. (Amended) Method according to claim 1, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

17. (Amended) Method according to claim 1, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

18. (Amended) Method according to claim 1, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

19. (Amended) Method according to claim 1, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

20. (Amended) Method according to claim 1, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

21. (Amended) Method according to claim 1, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

22. (Amended) Method according to claim 1, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

23. (Amended) Method according to claim 1, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

24. (Amended) Method according to claim 1, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

25. (Amended) Method according to claim 1, where the coat protein is derived from polyomavirus.

26. (Amended) Method according to claim 1, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

27. (Amended) Method according to claim 1, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

28. (Amended) Method according to claim 1, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

29. (Amended) Method according to claim 1, where the cell is a vertebrate cell or a human cell.

30. (Amended) Method according to claim 1, where at least two dsRNAs which differ from each other are introduced into the cell, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

31. (Amended) Method according to claim 1, where one of the target genes is the PKR gene.

34. (Amended) Medicament according to claim 32, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

35. (Amended) Medicament according to claim 32, where the target gene can be expressed in eukaryotic cells.

36. (Amended) Medicament according to claim 32, where the target gene is selected from

the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

37. (Amended) Medicament according to claim 32, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

38. (Amended) Medicament according to claim32, where the target gene is part of a virus or viroid.

41. (Amended) Medicament according to claim 32, where segments of the dsRNA are in double-stranded form.

42. (Amended) Medicament according to claim 32, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

43. (Amended) Medicament according to claim 32, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

44. (Amended) Medicament according to claim 32, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

45. (Amended) Medicament according to claim 32, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

46. (Amended) Medicament according to claim 32, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

47. (Amended) Medicament according to claim 32, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

48. (Amended) Medicament according to claim 32, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

49. (Amended) Medicament according to claim 32, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

50. (Amended) Medicament according to claim 32, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

51. (Amended) Medicament according to claim 32, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

52. (Amended) Medicament according to claim 32, where the chemical linkage are [sic] triple-helix bonds provided at the ends of the double-stranded structure.

53. (Amended) Medicament according to claim 32, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

54. (Amended) Medicament according to claim 32, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

55. (Amended) Medicament according to claim 32, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

56. (Amended) Medicament according to claim 32, where the coat protein is derived from the polyomavirus.

57. (Amended) Medicament according to claim 32, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

58. (Amended) Medicament according to claim 32, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

59. (Amended) Medicament according to claim 32, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

60. (Amended) Medicament according to claim 32, where the cell is a vertebrate cell or a human cell.

61. (Amended) Medicament according to claim 32, where at least two dsRNAs which differ from each other are contained in the medicament, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

65. (Amended) Active ingredient according to claim 63, where segments of the dsRNA are in double-stranded form.

66. (Amended) Active ingredient according to claim 63, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

67. (Amended) Active ingredient according to claim 63, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

68. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

69. (Amended) Active ingredient according to claim 63, where the chemical linkage is

generated at at least one, preferably both, ends of the double-stranded structure.

70. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

71. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

72. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

73. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

74. (Amended) Active ingredient according to claim 63, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

75. (Amended) Active ingredient according to claim 63, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

76. (Amended) Active ingredient according to claim 63, where the chemical linkage are triple-helix bonds provided at the ends of the double-stranded structure.

77. (Amended) Active ingredient according to claim 63, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

78. (Amended) Active ingredient according to claim 63, where at least one nucleotides at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

79. (Amended) Active ingredient according to claim 63, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

80. (Amended) Active ingredient according to claim 63, where at least two dsRNAs which differ from each other are contained in the active ingredient, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

83. (Amended) Use according to claim 81, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

84. (Amended) Use according to claim 81, where the target gene can be expressed in eukaryotic cells.

85. (Amended) Use according to claim 81, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

86. (Amended) Use according to claim 81, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

87. (Amended) Use according to claim 81, where the target gene is part of a virus or viroid.

90. (Amended) Use according to claim 81, where segments of the dsRNA are in double-stranded form.

91. (Amended) Use according to claim 81, where the ends of the dsRNA are modified in

order to counteract degradation in the cell or dissociation into the single strands.

92. (Amended) Use according to claim 81, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

93. (Amended) Use according to claim 81, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

94. (Amended) Use according to claim 81, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

95. (Amended) Use according to claim 81, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

96. (Amended) Use according to claim 81, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

97. (Amended) Use according to claim 81, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

98. (Amended) Use according to claim 81, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

99. (Amended) Use according to claim 81, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

100. (Amended) Use according to claim 81, where the chemical linkage is formed by thiophosphoryl groups attached to the ends of the double-stranded structure.

101. (Amended) Use according to claim 81, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

102. (Amended) Use according to claim 81, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

103. (Amended) Use according to claim 81, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

104. (Amended) Use according to claim 81, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

105. (Amended) Use according to claim 81, where the coat protein is derived from polyomavirus.

106. (Amended) Use according to claim 81, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

107. (Amended) Use according to claim 81, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

108. (Amended) Use according to claim 81, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

109. (Amended) Use according to claim 81, where the cell is a vertebrate cell or a human cell.

110. (Amended) Use according to claim 81, where at least two dsRNAs which differ from each other are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

112. (Amended) Use according to claim 81, where the medicament is injectable into the bloodstream or into the interstitium of the organism to undergo therapy.

113. (Amended) Use according to claim 81, where the dsRNA is taken up into bacteria or microorganisms.

116. (Amended) Use according to claim 114, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

117. (Amended) Use according to claim 114, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

118. (Amended) Use according to claim 114, where the target gene is part of a virus or viroid.

121. (Amended) Use according to claim 114, where segments of the dsRNA are in double-stranded form.

122. (Amended) Use according to claim 114, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

123. (Amended) Use according to claim 114, where the cell is a vertebrate cell or a human cell.

124. (Amended) Use according to claim 114, where at least two dsRNAs which differ from each other are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

125. (Amended) Use according to claim 124, where one of the target genes is the PKR gene.

REMARKS

The application has been amended to conform to U.S. practice. The claims have been amended to eliminate multiple dependency.

STATEMENT UNDER 37 CFR 1.821(f)

I, John P. Murtaugh, hereby state that the information recorded in computer readable form is identical to the written sequence listing.

Please charge any fees and credit any overpayments under 37 CFR 1.16 and 1.17 during the entire pendency of this application to our Deposit Account No. 16-0820, Order No. 33796.

If any fees are required by this communication, please charge such fees to our Deposit Account No. 16-0820, Order No. 33796.

Respectfully submitted,

PEARNE & GORDON LLP

By John P. Murtaugh

John P. Murtaugh. Reg. No. 34226

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(216) 579-1700

Date: 7-20-01

INDICATION OF REVISIONS TO THE CLAIMS
IN U.S. NATIONAL PHASE OF PCT/DE00/00244

3. (Amended) Method according to [either of the preceding claims] claim 1, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

4. (Amended) Method according to [one of the preceding claims] claim 1, where the target gene is expressed in eukaryotic cells.

5. (Amended) Method according to [one of the preceding claims] claim 1, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

6. (Amended) Method according to [one of the preceding claims] claim 1, where the target gene is expressed in pathogenic organisms, preferably in plasmodia.

7. (Amended) Method according to [one of the preceding claims] claim 1, where the target gene is part of a virus or viroid.

10. (Amended) Method according to [one of the preceding claims] claim 1, where segments of the dsRNA are in double-stranded form.

11. (Amended) Method according to [one of the preceding claims] claim 1, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

12. (Amended) Method according to [one of the preceding claims] claim 1, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide

pairs, is increased by at least one, preferably two, further chemical linkage(s).

13. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

14. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

15. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

16. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

17. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

18. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

19. (Amended) Method according to [one of the preceding claims] claim 1, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

20. (Amended) Method according to [one of the preceding claims] claim 1, where the

chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

21. (Amended) Method according to [one of the preceding claims] claim 1, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

22. (Amended) Method according to [one of the preceding claims] claim 1, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

23. (Amended) Method according to [one of the preceding claims] claim 1, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

24. (Amended) Method according to [one of the preceding claims] claim 1, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

25. (Amended) Method according to [one of the preceding claims] claim 1, where the coat protein is derived from polyomavirus.

26. (Amended) Method according to [one of the preceding claims] claim 1, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

27. (Amended) Method according to [one of the preceding claims] claim 1, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

28. (Amended) Method according to [one of the preceding claims] claim 1, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

29. (Amended) Method according to [one of the preceding claims] claim 1, where the cell is a vertebrate cell or a human cell.

30. (Amended) Method according to [one of the preceding claims] claim 1, where at least two dsRNAs which differ from each other are introduced into the cell, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

31. (Amended) Method according to [one of the preceding claims] claim 1, where one of the target genes is the PKR gene.

34. (Amended) Medicament according to [either of claims 32 or 33] claim 32, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

35. (Amended) Medicament according to [one of claims 32 to 34] claim 32, where the target gene can be expressed in eukaryotic cells.

36. (Amended) Medicament according to [one of claims 32 to 35] claim 32, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

37. (Amended) Medicament according to [one of claims 32 to 36] claim 32, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

38. (Amended) Medicament according to [one of claims 32 to 37] claim32, where the target gene is part of a virus or viroid.

41. (Amended) Medicament according to [one of claims 32 to 40] claim 32, where segments of the dsRNA are in double-stranded form.

42. (Amended) Medicament according to [one of claims 32 to 40] claim 32, where the

ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

43. (Amended) Medicament according to [one of claims 32 to 42] claim 32, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

44. (Amended) Medicament according to [one of claims 32 to 43] claim 32, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

45. (Amended) Medicament according to [one of claims 32 to 44] claim 32, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

46. (Amended) Medicament according to [one of claims 32 to 45] claim 32, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

47. (Amended) Medicament according to [one of claims 32 to 46] claim 32, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

48. (Amended) Medicament according to [one of claims 32 to 47] claim 32, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

49. (Amended) Medicament according to [one of claims 32 to 48] claim 32, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

50. (Amended) Medicament according to [one of claims 32 to 49] claim 32, where at

least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

51. (Amended) Medicament according to [one of claims 32 to 50] claim 32, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

52. (Amended) Medicament according to [one of claims 32 to 51] claim 32, where the chemical linkage are [sic] triple-helix bonds provided at the ends of the double-stranded structure.

53. (Amended) Medicament according to [one of claims 32 to 52] claim 32, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

54. (Amended) Medicament according to [one of claims 32 to 53] claim 32, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

55. (Amended) Medicament according to [one of claims 32 to 54] claim 32, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

56. (Amended) Medicament according to [one of claims 32 to 55] claim 32, where the coat protein is derived from the polyomavirus.

57. (Amended) Medicament according to [one of claims 32 to 56] claim 32, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

58. (Amended) Medicament according to [one of claims 32 to 57] claim 32, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of

the capsid or capsid-type structure.

59. (Amended) Medicament according to [one of claims 32 to 58] claim 32, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

60. (Amended) Medicament according to [one of claims 32 to 59] claim 32, where the cell is a vertebrate cell or a human cell.

61. (Amended) Medicament according to [one of claims 32 to 60] claim 32, where at least two dsRNAs which differ from each other are contained in the medicament, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

65. (Amended) Active ingredient according to claim 63 [or 64], where segments of the dsRNA are in double-stranded form.

66. (Amended) Active ingredient according to [one of claims 63 to 65] claim 63, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

67. (Amended) Active ingredient according to [one of claims 63 to 66] claim 63, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

68. (Amended) Active ingredient according to [one of claims 63 to 67] claim 63, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

69. (Amended) Active ingredient according to [one of claims 63 to 68] claim 63, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

70. (Amended) Active ingredient according to [one of claims 63 to 69] claim 63, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

71. (Amended) Active ingredient according to [one of claims 63 to 70] claim 63, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

72. (Amended) Active ingredient according to [one of claims 63 to 71] claim 63, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

73. (Amended) Active ingredient according to [one of claims 63 to 72] claim 63, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

74. (Amended) Active ingredient according to [one of claims 63 to 73] claim 63, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

75. (Amended) Active ingredient according to [one of claims 63 to 74] claim 63, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

76. (Amended) Active ingredient according to [one of claims 63 to 75] claim 63, where the chemical linkage are triple-helix bonds provided at the ends of the double-stranded structure.

77. (Amended) Active ingredient according to [one of claims 63 to 76] claim 63, where at

least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

78. (Amended) Active ingredient according to [one of claims 63 to 77] claim 63, where at least one nucleotides at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

79. (Amended) Active ingredient according to [one of claims 63 to 78] claim 63, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

80. (Amended) Active ingredient according to [one of claims 63 to 79] claim 63, where at least two dsRNAs which differ from each other are contained in the active ingredient, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

83. (Amended) Use according to [either of claims 81 or 82] claim 81, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

84. (Amended) Use according to [one of claims 81 to 83] claim 81, where the target gene can be expressed in eukaryotic cells.

85. (Amended) Use according to [one of claims 81 to 84] claim 81, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

86. (Amended) Use according to [one of claims 81 to 85] claim 81, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

87. (Amended) Use according to [one of claims 81 to 86] claim 81, where the target gene is part of a virus or viroid.

90. (Amended) Use according to [one of claims 81 to 89] claim 81, where segments of the dsRNA are in double-stranded form.

91. (Amended) Use according to [one of claims 81 to 90] claim 81, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

92. (Amended) Use according to [one of claims 81 to 91] claim 81, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

93. (Amended) Use according to [one of claims 81 to 92] claim 81, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

94. (Amended) Use according to [one of claims 81 to 93] claim 81, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

95. (Amended) Use according to [one of claims 81 to 94] claim 81, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

96. (Amended) Use according to [one of claims 81 to 95] claim 81, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

97. (Amended) Use according to [one of claims 81 to 96] claim 81, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

98. (Amended) Use according to [one of claims 81 to 97] claim 81, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

99. (Amended) Use according to [one of claims 81 to 98] claim 81, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

100. (Amended) Use according to [one of claims 81 to 99] claim 81, where the chemical linkage is formed by thiophosphoryl groups attached to the ends of the double-stranded structure.

101. (Amended) Use according to [one of claims 81 to 100] claim 81, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

102. (Amended) Use according to [one of claims 81 to 101] claim 81, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

103. (Amended) Use according to [one of claims 81 to 102] claim 81, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

104. (Amended) Use according to [one of claims 81 to 103] claim 81, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

105. (Amended) Use according to [one of claims 81 to 104] claim 81, where the coat protein is derived from polyomavirus.

106. (Amended) Use according to [one of claims 81 to 105] claim 81, where the coat

protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

107. (Amended) Use according to [one of claims 81 to 106] claim 81, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

108. (Amended) Use according to [one of claims 81 to 107] claim 81, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

109. (Amended) Use according to [one of claims 81 to 108] claim 81, where the cell is a vertebrate cell or a human cell.

110. (Amended) Use according to [one of claims 81 to 109] claim 81, where at least two dsRNAs which differ from each other are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

112. (Amended) Use according to [one of claims 81 to 111] claim 81, where the medicament is injectable into the blood-stream or into the interstitium of the organism to undergo therapy.

113. (Amended) Use according to [one of claims 81 to 112] claim 81, where the dsRNA is taken up into bacteria or micro-organisms.

116. (Amended) Use according to claim 114 [or 115], where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

117. (Amended) Use according to [one of claims 114 to 116] claim 114, where the target gene can be expressed in patho-genic organisms, preferably in plasmodia.

118. (Amended) Use according to [one of claims 114 to 117] claim 114, where the target gene is part of a virus or viroid.

121. (Amended) Use according to [one of claims 114 to 120] claim 114, where segments of the dsRNA are in double-stranded form.

122. (Amended) Use according to [one of claims 114 to 121] claim 114, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

123. (Amended) Use according to [one of claims 114 to 122] claim 114, where the cell is a vertebrate cell or a human cell.

124. (Amended) Use according to [one of claims 114 to 123] claim 114, where at least two dsRNAs which differ from each other are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

125. (Amended) Use according to claim [125] 124, where one of the target genes is the PKR gene.

Method and medicament for inhibiting the expression of a given gene

The invention relates to methods in accordance with the preambles of claims 1 and 2. It furthermore relates to a medicament and to a use of double-stranded oligoribonucleotides and to a vector encoding them.

Such a method is known from WO 99/32619, which was unpublished at the priority date of the present invention. The known process aims at inhibiting the expression of genes in cells of invertebrates. To this end, the double-stranded oligoribonucleotide must exhibit a sequence which is identical with the target gene and which has a length of at least 50 bases. To achieve efficient inhibition, the identical sequence must be 300 to 1 000 base pairs in length. Such an oligoribonucleotide is complicated to prepare.

DE 196 31 919 C2 describes an antisense RNA with specific secondary structures, the antisense RNA being present in the form of a vector encoding it. The antisense RNA takes the form of an RNA molecule which is complementary to regions of the mRNA. Inhibition of the gene expression is caused by binding to these regions. This inhibition can be employed in particular for the diagnosis and/or therapy of diseases, for example tumor diseases or viral infections. - The disadvantage is that the antisense RNA must be introduced into the cell in an amount which is at least as high as the amount of the mRNA. The known antisense methods are not particularly effective.

US 5,712,257 discloses a medicament comprising mismatched double-stranded RNA (dsRNA) and bioactive mismatched fragments of dsRNA in the form of a ternary complex together with a surfactant. The dsRNA used for this purpose consists of synthetic nucleic acid single strands without defined base sequence. The single

strands undergo irregular base pairing, also known as "non-Watson-Crick" base pairing, giving rise to mismatched double strands. The known dsRNA is used to inhibit the amplification of retroviruses such as HIV. Amplification of the virus can be inhibited when non-sequence-specific dsRNA is introduced into the cells. This leads to the induction of interferon, which is intended to inhibit viral amplification. The inhibitory effect, or the activity, of this method is poor.

It is known from Fire, A. et al., NATURE, Vol. 391, pp. 806 that dsRNA whose one strand is complementary in segments to a nematode gene to be inhibited inhibits the expression of this gene highly efficiently. It is believed that the particular activity of the dsRNA used in nematode cells is not due to the antisense principle but possibly on catalytic properties of the dsRNA, or enzymes induced by it. - Nothing is mentioned in this paper on the activity of specific dsRNA with regard to inhibiting the gene expression, in particular in mammalian and human cells.

The object of the present invention is to do away with the disadvantages of the prior art. In particular, it is intended to provide as effective as possible a method, medicament or use for the preparation of a medicament, which method, medicament or use is capable of causing particularly effective inhibition of the expression of a given target gene.

This object is achieved by the features of claims 1, 2, 37, 38 and 74 and 75. Advantageous embodiments can be seen from claims 3 to 36, 39 to 73 and 76 to 112.

In accordance with the method-oriented inventions, it is provided in each case that the region I which is complementary to the target gene exhibits not more than 49 successive nucleotide pairs.

Provided in accordance with the invention are an oligoribonucleotide or a vector encoding therefor. At least segments of the oligoribonucleotide exhibit a defined nucleotide sequence. The defined segment may be limited to the complementary region I. However, it is also possible that all of the double-stranded oligoribonucleotide exhibits a defined nucleotide sequence.

Surprisingly, it has emerged that an effective inhibition of the expression of the target gene can be achieved even when the complementary region I is not more than 49 base pairs in length. The procedure of providing such oligoribonucleotides is less complicated.

In particular, dsRNA with a length of over 50 nucleotide pairs induces certain cellular mechanisms, for example the dsRNA-dependent protein kinase or the 2-5A system, in mammalian and human cells. This leads to the disappearance of the interference effect mediated by the dsRNA which exhibits a defined sequence. As a consequence, protein biosynthesis in the cell is blocked. The present invention overcomes this disadvantage in particular.

Furthermore, the uptake of dsRNA with short chain lengths into the cell or into the nucleus is facilitated markedly over longer-chain dsRNAs.

It has proved advantageous for the dsRNA or the vector to be present packaged into micellar structures, preferably in liposomes. The dsRNA or the vector can likewise be enclosed in viral natural capsids or in chemically or enzymatically produced artificial capsids or structures derived therefrom. - The abovementioned features make it possible to introduce the dsRNA or the vector into given target cells.

In a further aspect, the dsRNA has 10 to 1 000, preferably 15 to 49, base pairs. Thus, the dsRNA can be longer than the region I, which is complementary to the target gene. The complementary region I can be located at the terminus or inserted into the dsRNA. Such dsRNA or a vector provided for coding the same can be produced synthetically or enzymatically by customary methods.

The gene to be inhibited is expediently expressed in eukaryotic cells. The target gene can be selected from the following group: oncogene, cytokin gene, Id protein gene, developmental gene, prion gene. It can also be expressed in pathogenic organisms, preferably in plasmodia. It can be part of a virus or viroid which is preferably pathogenic to humans. - The method proposed makes it possible to produce compositions for the therapy of genetically determined diseases, for example cancer, viral diseases or Alzheimer's disease.

The virus or viroid can also be a virus or viroid which is pathogenic to animals or plant-pathogenic. In this case, the method according to the invention also permits the provision of compositions for treating animal or plant diseases.

In a further aspect, segments of the dsRNA are designed as double-stranded. A region II which is complementary within the double-stranded structure is formed by two separate RNA single strands or by autocomplementary regions of a topologically closed RNA single strand which is preferably in circular form.

The ends of the dsRNA can be modified to counteract degradation in the cell or dissociation into the single strands. Dissociation takes place in particular when low concentrations or short chain lengths are used. To inhibit dissociation in a particularly effective fashion, the cohesion of the complementary region II,

which is caused by the nucleotide pairs, can be increased by at least one, preferably two, further chemical linkage(s). - A dsRNA according to the invention whose dissociation is reduced exhibits greater stability to enzymatic and chemical degradation in the cell or in the organism.

The complementary region II can be formed by autocomplementary regions of an RNA hairpin loop, in particular when using a vector according to the invention. To afford protection from degradation, it is expedient for the nucleotides to be chemically modified in the loop region between the double-stranded structure.

The chemical linkage is expediently formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination. In an especially advantageous aspect, it can be formed at at least one, preferably both, end(s) of the complementary region II.

It has furthermore proved to be advantageous for the chemical linkage to be formed by one or more linkage groups, the linkage groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains. The chemical linkage can also be formed by purine analogs used in place of purines in the complementary regions II. It is also advantageous for the chemical linkage to be formed by azabenzene units introduced into the complementary regions II. Moreover, it can be formed by branched nucleotide analogs used in place of nucleotides in the complementary regions II.

It has proved expedient to use at least one of the following groups for generating the chemical linkage: methylene blue; bifunctional groups, preferably

bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene. The chemical linkage can furthermore be formed by thiophosphoryl groups provided at the ends of the double-stranded region. The chemical linkage at the ends of the double-stranded region is preferably formed by triple-helix bonds.

The chemical linkage can expediently be induced by ultraviolet light.

The nucleotides of the dsRNA can be modified. This counteracts the activation, in the cell, of a double-stranded-RNA-dependent protein kinase, PKR. Advantageously, at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the complementary region II is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group. At least one nucleotide in at least one strand of the complementary region II can also be a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C methylene bridge. Advantageously, several nucleotides are locked nucleotides.

A further especially advantageous embodiment provides that the dsRNA or the vector is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically. The coat protein can be derived from polyomavirus. The coat protein can contain the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2). The use of such coat proteins is known from, for example, DE 196 18 797 A1, whose disclosure is herewith incorporated. - The abovementioned features considerably facilitate the introduction of the dsRNA or of the vector into the cell.

When a capsid or capsid-type structure is formed from the coat protein, one side preferably faces the interior of the capsid or capsid-type structure. The construct formed is particularly stable.

The dsRNA can be complementary to the primary or processed RNA transcript of the target gene. - The cell can be a vertebrate cell or a human cell.

At least two dsRNAs which differ from each other or at least one vector encoding them can be introduced into the cell, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes. This makes it possible simultaneously to inhibit the expression of at least two different target genes. In order to suppress, in the cell, the expression of a double-stranded-RNA-dependent protein kinase, PKR, one of the target genes is advantageously the PKR gene. This allows effective suppression of the PKR activity in the cell.

The invention furthermore provides a medicament with at least one oligoribonucleotide with double-stranded structure (dsRNA) for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region I where at least segments are complementary to the target gene. - Surprisingly, it has emerged that such a dsRNA is suitable as medicament for inhibiting the expression of a given gene in mammalian cells. In comparison with the use of single-stranded oligoribonucleotides, the inhibition is already caused at concentrations which are lower by at least one order of magnitude. The medicament according to the invention is highly effective. Lesser side effects can be expected.

The invention furthermore provides a medicament with at least one vector for coding at least one oligoribonucleotide with double-stranded structure

(dsRNA) for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region I where at least segments are complementary to the target gene. - The medicament proposed exhibits the abovementioned advantages. By using a vector, in particular production costs can be reduced.

In a particularly advantageous embodiment, the complementary region I has not more than 49 successive nucleotide pairs. - Surprisingly, it has emerged that an effective inhibition of the expression of the target gene can be achieved even when the complementary region I is not more than 49 base pairs in length. The procedure of providing such oligoribonucleotides is less complicated.

The invention furthermore provides a use of an oligoribonucleotide with double-stranded structure (dsRNA) for preparing a medicament for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region I where at least segments are complementary to the target gene. - Surprisingly, such a dsRNA is suitable for preparing a medicament for inhibiting the expression of a given gene. Compared with the use of single-stranded oligoribonucleotides, the inhibition is already caused at concentrations which are lower by one order of magnitude when using dsRNA. The use according to the invention thus makes possible the preparation of particularly effective medicaments.

The invention furthermore provides the use of a vector for coding at least one oligoribonucleotide with double-stranded structure (dsRNA) for preparing a medicament for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region I where at least segments are complementary to this target gene. - The use of a vector makes possible a particularly effective gene therapy.

With regard to advantageous embodiments of the medicament and of the use, reference is made to the description of the above features.

Use examples of the invention are illustrated in greater detail hereinbelow with reference to the figures, in which:

Fig. 1 shows the schematic representation of a plasmid for the *in vitro* transcription with T7- and SP6-polymerase,

Fig. 2 shows RNA following electrophoresis on an 8% polyacrylamide gel and staining with ethidium bromide,

Fig. 3 shows a representation of radioactive RNA transcripts following electrophoresis on an 8% polyacrylamide gel with 7 M urea by means of an instant imager, and

Figs. 4a - e show Texas Red and YFP fluorescence in murine fibroblasts.

Use example 1:

The inhibition of transcription was detected by means of sequence homologous dsRNA in an *in vitro* transcription system with a nuclear extract from human HeLa cells. The DNA template for this experiment was plasmid pCMV1200 which had been linearized by means of *Bam*HI.

Generation of the template plasmids:

The plasmid shown in fig. 1 was constructed for use in the enzymatic synthesis of the dsRNA. To this end, a polymerase chain reaction (PCR) with the "positive control DNA" of the HelaScribe® Nuclear Extract *in vitro* transcription kit by Promega, Madison, USA, as

DNA template was first carried out. One of the primers used contained the sequence of an *Eco*RI cleavage site and of the T7 RNA polymerase promoter as shown in sequence listing No. 1. The other primer contained the sequence of a *Bam*HI cleavage site and of the SP6 RNA polymerase promoter as shown in sequence listing No. 2. In addition, the two primers had, at the 3' ends, regions which were identical with or complementary to the DNA template. The PCR was carried out by means of the "*Taq PCR Core Kits*" by Qiagen, Hilden, Germany, following the manufacturer's instructions. 1.5 mM $MgCl_2$, in each case 200 µM dNTP, in each case 0.5 µM primer, 2.5 U *Taq* DNA polymerase and approximately 100 ng of "positive control DNA" were employed as template in PCR buffer in a volume of 100 µl. After initial denaturation of the template DNA by heating for 5 minutes at 94°C, amplification was carried out in 30 cycles of denaturation for in each case 60 seconds at 94°C, annealing for 60 seconds at 5°C below the calculated melting point of the primers and polymerization for 1.5-2 minutes at 72°C. After a final polymerization of 5 minutes at 72°C, 5 µl of the reaction were analyzed by agarose-gel electrophoresis. The length of the DNA fragment amplified thus was 400 base pairs, 340 base pairs corresponding to the "positive control DNA". The PCR product was purified, hydrolyzed with *Eco*RI and *Bam*HI and, after repurification, employed in the ligation together with a pUC18 vector which had also been hydrolyzed by *Eco*RI and *Bam*HI. *E. coli* XL1-blue was then transformed. The plasmid obtained (pCMV5) carries a DNA fragment whose 5' end is flanked by the T7 promoter and whose 3' end is flanked by the SP6 promoter. By linearizing the plasmid with *Bam*HI, it can be employed *in vitro* with the T7-RNA polymerase for the run-off transcription of a single-stranded RNA which is 340 nucleotides in length and shown in sequence listing No. 3. If the plasmid is linearized with *Eco*RI, it can be employed for the run-off transcription with SP6 RNA polymerase,

giving rise to the complementary strand. In accordance with the method outlined hereinabove, an RNA 23 nucleotides in length was also synthesized. To this end, a DNA shown in sequence listing No. 4 was ligated with the pUC18 vector via the *Eco*RI and *Bam*HI cleavage sites.

Plasmid pCMV1200 was constructed as DNA template for the *in-vitro* transcription with HeLa nuclear extract. To this end, a 1 191 bp EcoRI/BamHI fragment of the positive control DNA contained in the HeLaScribe® Nuclear Extract *in vitro* transcription kit was amplified by means of PCR. The amplified fragment encompasses the 828 bp "immediate early" CMV promoter and a 363 bp transcribable DNA fragment. The PCR product was ligated to the vector pGEM-T via "T-overhang" ligation. A BamHI cleavage site is located at the 5' end of the fragment. The plasmid was linearized by hydrolysis with BamHI and used as template in the run-off transcription.

In-vitro transcription of the complementary single strands:

pCMV5 plasmid DNA was linearized with *Eco*RI or *Bam*HI. It was used as DNA template for an *in-vitro* transcription of the complementary RNA single strands with SP6 and T7 RNA polymerase, respectively. The "Riboprobe *in vitro* Transcription" system by Promega, Madison, USA, was employed for this purpose. Following the manufacturer's instructions, 2 µg of linearized plasmid DNA were incubated in 100 µl of transcription buffer and 40 U T7 or SP6 RNA polymerase for 5-6 hours at 37°C. The DNA template was subsequently degraded by addition of 2.5 µl of RNase-free DNase RQ1 and incubation for 30 minutes at 37°C. The transcription reaction was made up to 300 µl with H_2O and purified by phenol extraction. The RNA was precipitated by addition of 150 µl of 7 M ammonium acatate [sic] and 1 125 µl of

ethanol and stored at -65°C until used for the hybridization.

Generation of the RNA double strands:

For the hybridization, 500 µl of the single-stranded RNA which had been stored in ethanol and precipitated were spun down. The resulting pellet was dried and taken up in 30 µl of PIPES buffer, pH 6.4 in the presence of 80% formamide, 400 mM NaCl and 1 mM EDTA. In each case 15 µl of the complementary single strands were combined and heated for 10 minutes at 85°C. The reactions were subsequently incubated overnight at 50°C and cooled to room temperature.

Only approximately equimolar amounts of the two single strands were employed in the hybridization. This is why the dsRNA preparations contained single-stranded RNA (ssRNA) as contaminant. In order to remove these ssRNA contaminants, the reactions were treated, after hybridization, with the single-strand-specific ribonucleases bovine pancreatic RNase A and *Aspergillus oryzae* RNase T1. RNase A is an endoribonuclease which is specific for pyrimidines. RNase T1 is an endoribonuclease which preferentially cleaves at the 3' side of guanosines. dsRNA is no substrate for these ribonucleases. For the RNase treatment, the reactions in 300 µl of Tris, pH 7.4, 300 mM NaCl and 5 mM EDTA were treated with 1.2 µl of RNaseA at a concentration of 10 mg/ml and 2 µl of RNaseT1 at a concentration of 290 µg/ml. The reactions were incubated for 1.5 hours at 30°C. Thereupon, the RNases were denatured by addition of 5 µl of proteinase K at a concentration of 20 mg/ml and 10 µl of 20% SDS and incubation for 30 minutes at 37°C. The dsRNA was purified by phenol extraction and precipitated with ethanol. To verify the completeness of the RNase digestion, two control reactions were treated with ssRNA analogously to the hybridization reactions.

The dried pellet was taken up in 15 µl of TE buffer, pH 6.5, and subjected to native polyacrylamide gel electrophoresis on an 8% gel. The acrylamide gel was subsequently stained in an ethidium bromide solution and washed in a water bath. Fig. 2 shows the RNA which had been visualized in a UV transilluminator. The *sense* RNA which had been applied to lane 1 and the *antisense* RNA which had been applied to lane 2 showed a different migration behavior under the chosen conditions than the dsRNA of the hybridization reaction which had been applied to lane 3. The RNase-treated *sense* RNA and *antisense* RNA which had been applied to lanes 4 and 5, respectively, produced no visible band. This shows that the single-stranded RNAs had been degraded completely. The RNase-treated dsRNA of the hybridization reaction which had been applied to lane 6 is resistant to RNase treatment. The band which migrates faster in the native gel in comparison with the dsRNA applied to lane 3 results from dsRNA which is free from ssRNA. In addition to the dominant main band, weaker bands which migrate faster are observed after the RNase treatment.

In-vitro transcription test with human nuclear extract:

Using the HeLaScribe® Nuclear Extract *in vitro* transcription kit by Promega, Madison, USA, the transcription efficiency of the abovementioned DNA fragment which is present in plasmid pCMV1200 and homologous to the "positive control DNA" was determined in the presence of the dsRNA (dsRNA-CMV5) with sequence homology. Also, the effect of the dsRNA without sequence homology, which corresponds to the yellow fluorescent protein (YFP) gene (dsRNA-YRP), was studied. This dsRNA had been generated analogously to the dsRNA with sequence homology. The sequence of a strand of this dsRNA can be found in sequence listing No. 5. Plasmid pCMV1200 was used as template for the run-off transcription. It carries the "immediate early" cytomegalovirus promoter which is recognized by the eukaryotic RNA polymerase II, and a transcribable DNA

fragment. Transcription was carried out by means of the HeLa nuclear extract, which contains all the proteins which are necessary for transcription. By addition of [·-^{32}P]rGTP to the transcription reaction, radiolabeled transcript was obtained. The [·-^{32}P]rGTP used had a specific activity of 400 Ci/mmol, 10 mCi/ml. 3 mM $MgCl_2$, in each case 400 µM rATP, rCTP, rUTP, 16 µM rGTP, 0.4 µM [·-^{32}P]rGTP and depending on the experiment 1 fmol of linearized plasmid DNA and various amounts of dsRNA in transcription buffer were employed per reaction. Each batch was made up to a volume of 8.5 µl with H_2O. The reactions were mixed carefully. To start the transcription, 4 U HeLa nuclear extract in a volume of 4 µl were added and incubated for 60 minutes at 30°C. The reaction was stopped by addition of 87.5 µl of quench mix which had been warmed to 30°C. To remove the proteins, the reactions were treated with 100 µl of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v) saturated with TE buffer, pH 5.0, and the reactions were mixed vigorously for 1 minute. For phase separation, the reactions were spun for approximately 1 minute at 12 000 rpm and the top phase was transferred into a fresh reaction vessel. Each reaction was treated with 250 µl of ethanol. The reactions were mixed thoroughly and incubated for at least 15 minutes on dry ice/methanol. To precipitate the RNA, the reactions were spun for 20 minutes at 12 000 rpm and 40°C. The supernatant was discarded. The pellet was dried in vacuo for 15 minutes and resuspended in 10 µl of H_2O. Each reaction was treated with 10 µl of denaturing loading buffer. The free GTP was separated from the transcript formed by means of denaturing polyacrylamide gel electrophoresis on an 8% gel with 7 M urea. The RNA transcripts formed upon transcription with HeLa nuclear extract, in denaturing loading buffer, were heated for 10 minutes at 90°C and 10 µl aliquots were applied immediately to the freshly washed pockets. The electrophoresis was run at 40 mA. The amount of the radioactive ssRNA formed upon

transcription was analyzed after electrophoresis with the aid of an *Instant Imager*.

Fig. 3 shows the radioactive RNA from a representative test, shown by means of the *Instant Imager*. Samples obtained from the following transcription reactions were applied:

Lane 1: without template DNA, without dsRNA;
Lane 1: 50 ng of template DNA, without dsRNA;
Lane 3: 50 ng of template DNA, 0.5 µg of dsRNA YFP;
Lane 4: 50 ng of template DNA, 1.5 µg of dsRNA YFP;
Lane 5: 50 ng of template DNA, 3 µg of dsRNA YFP;
Lane 6: 50 ng of template DNA, 5 µg of dsRNA YFP;
Lane 7: without template DNA, 1.5 dsRNA YFP;
Lane 8: 50 ng of template DNA, without dsRNA;
Lane 9: 50 ng of template DNA, 0.5 µg of dsRNA CMV5;
Lane 10: 50 ng of template DNA, 1.5 µg of dsRNA CMV5;
Lane 11: 50 ng of template DNA, 3 µg of dsRNA CMV5;
Lane 12: 50 ng of template DNA, 5 µg of dsRNA CMV5;

It emerged that the amount of transcript was reduced markedly in the presence of dsRNA with sequence homology in comparison with the control reaction without dsRNA and with the reactions with dsRNA YFP without sequence homology. The positive control in lane 2 shows that radioactive transcript was formed upon the *in-vitro* transcription with HeLa nuclear extract. The reaction is used for comparison with the transcription reactions which had been incubated in the presence of dsRNA. Lanes 3 to 6 show that the addition of non-sequentially-specific dsRNA YFP had no effect on the amount of transcript formed. Lanes 9 to 12 show that the addition of an amount of between 1.5 and 3 µg of sequentially-specific dsRNA CMV5 leads to a reduction in the amount of transcript formed. In order to exclude that the effects observed are based not on the dsRNA but on any contamination which might have been carried along accidentally during the preparation of the dsRNA,

a further control was carried out. Single-stranded RNA was transcribed as described above and subsequently subjected to the RNase treatment. It was demonstrated by means of native polyacrylamide gel electrophoresis that the ssRNA had been degraded completely. This reaction was subjected to phenol extraction and ethanol precipitation and subsequently taken up in PE buffer, as were the hybridization reactions. This gave a sample which contained no RNA but had been treated with the same enzymes and buffers as the dsRNA. Lane 8 shows that the addition of this sample had no effect on transcription. The reduction of the transcript upon addition of sequence-specific dsRNA can therefore be ascribed unequivocally to the dsRNA itself. The reduction of the amount of transcript of a gene in the presence of dsRNA in a human transcription system indicates an inhibition of the expression of the gene in question. This effect can be attributed to a novel mechanism caused by the dsRNA.

Use example 2:

The test system used for these *in-vivo* experiments was the murine fibroblast cell line NIH3T3, ATCC CRL-1658. The YFP gene was introduced into the nuclei with the aid of microinjection. Expression of YFP was studied under the effect of simultaneously cotransfected dsRNA with sequence homology. This dsRNA YFP shows homology with the 5'-region of the YFP gene over a length of 315 bp. The nucleotide sequence of a strand of the dsRNA YRP is shown in sequence listing No. 5. Evaluation under the fluorescence microscope was carried out 3 hours after injection with reference to the greenish-yellow fluorescence of the YFP formed.

Construction of the template plasmid, and preparation of the dsRNA:

A plasmid was constructed following the same principle as described in use example 1 to act as template for the production of the YFP dsRNA by means of T7 and SP6

in-vitro transcription. Using the primer *Eco*_T7_YFP as shown in sequence listing No. 6 and *Bam*_SP6_YFP as shown in sequence listing No. 7, the desired gene fragment was amplified by PCR and used analogously to the above description for preparing the dsRNA. The dsRNA YFP obtained is identical to the dsRNA used in use example 1 as non-sequence-specific control.

A dsRNA linked chemically at the 3' end of the RNA as shown in sequence listing No. 8 to the 5' end of the complementary RNA via a C18 linker group was prepared (L-dsRNA). To this end, synthons modified by disulfide bridges were used. The 3'-terminal synthon is bound to the solid support via the 3' carbon with an aliphatic linker group via a disulfide bridge. In the 5'-terminal synthon of the complementary oligoribonucleotide which is complementary to the 3'-terminal synthon of the one oligoribonucleotide, the 5'-trityl protecting group is bound via a further aliphatic linker and a disulfide bridge. Following synthesis of the two single strands, removal of the protecting groups and hybridization of the complementary oligoribonucleotides, the thiol groups which form are brought into spatial vicinity. The single strands are linked to each other by oxidation via their aliphatic linkers and a disulfide bridge. This is followed by purification with the aid of HPLC.

Preparation of the cell cultures:

The cells were incubated in DMEM supplemented with 4.5 g/l glucose, 10% fetal bovine serum in culture dishes at 37°C under a 7.5% CO_2 atmosphere and passaged before reaching confluence. The cells were detached with trypsin/EDTA. To prepare for microinjection, the cells were transferred into Petri dishes and incubated further until microcolonies formed.

Microinjection:

For the microinjection, the culture dishes were removed from the incubator for approximately 10 minutes. Approximately 50 nuclei were injected singly per reaction within a marked area using the AIS microinjection system from Carl Zeiss, Göttingen, Germany. The cells were subsequently incubated for three more hours. For the microinjection, borosilicate glass capillaries from Hilgenberg GmbH, Malsfeld, Germany, with a diameter of less than 0.5 µm at the tip were prepared. The microinjection was carried out using a micromanipulator from Narishige Scientific Instrument Lab., Tokyo, Japan. The injection time was 0.8 seconds and the pressure was approximately 100 hPa. The transfection was carried out using the plasmid pCDNA YFP, which contains an approximately 800 bp *Bam*HI/*Eco*RI fragment with the YFP gene in vector pcDNA3. The samples injected into the nuclei contained 0.01 µg/µl of pCDNA-YFP and Texas Red coupled to dextran-70000 in 14 mM NaCl, 3 mM KCl, 10 mM KPO_4 [sic], ph 7.5. Approximately 100 pl of RNA with a concentration of 1 µM or, in the case of the L-dsRNA, 375 µM were additionally added.

The cells were studied under a fluorescence microscope with excitation with the light of the excitation wavelength of Texas Red, 568 nm, or of YFP, 488 nm. Individual cells were documented by means of a digital camers. Figures 4a-e show the result for NIH3T3 cells. In the cells shown in Fig. 4a, *sense*-YFP-ssRNA has been injected, in Fig. 4b *antisense*-YFP-ssRNA, in Fig. 4c dsRNA-YFP, in Fig. 4d no RNA and in Fig. 4e L-dsRNA.

The field on the left shows in each case the fluorescence of cells with excitation at 568 nm. The fluorescence of the same cells at an excitation of 488 nm is seen on the right. The Texas Red fluorescence of all the cells shown demonstrates that the injection solution had been applied successfully into the nuclei

and that cells with successful hits were still alive after three hours. Dead cells no longer showed Texas Red fluorescence.

The right fields of each of figures 4a and 4b show that YFP expression was not visibly inhibited when the single-stranded RNA was injected into the nuclei. The right field of Fig. 4c shows cells whose YFP fluorescence was no longer detectable after the injection of dsRNA-YFP. Fig. 4d shows cells into which no RNA had been injected, as control. The cell shown in fig. 4e shows YFP fluorescence which can no longer be detected owing to the injection of the L-dsRNA which shows regions with sequence homology to the YFP gene. This result demonstrates that even shorter dsRNAs can be used for specifically inhibiting gene expression in mammals when the double strands are stabilized by chemically linking the single strands.

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International Patent Application No. PCT/DE00/00244
of Dr Roland Kreutzer and Dr Stefan Limmer

New Patent Claims

1. Method for inhibiting the expression of a given target gene in a cell in vitro, where an oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands is introduced into the cell, where one strand of the dsRNA has a region which is complementary to the target gene,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

2. Method according to claim 1, where the dsRNA is enclosed by micellar structures, preferably by liposomes.

3. Method according to either of the preceding claims, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

4. Method according to one of the preceding claims, where the target gene is expressed in eukaryotic cells.

5. Method according to one of the preceding claims, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

6. Method according to one of the preceding claims, where the target gene is expressed in pathogenic organisms, preferably in plasmodia.

7. Method according to one of the preceding claims, where the target gene is part of a virus or viroid.

8. Method according to claim 7, where the virus is a virus or viroid which is pathogenic for humans.

9. Method according to claim 7, where the virus or viroid is a virus or viroid which is pathogenic for animals or phytopathogenic.

10. Method according to one of the preceding claims, where segments of the dsRNA are in double-stranded form.

11. Method according to one of the preceding claims, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

12. Method according to one of the preceding claims, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

13. Method according to one of the preceding claims, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

14. Method according to one of the preceding claims, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

15. Method according to one of the preceding claims, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

16. Method according to one of the preceding claims, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

17. Method according to one of the preceding claims, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

18. Method according to one of the preceding claims, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

19. Method according to one of the preceding claims, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene.

20. Method according to one of the preceding claims, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

21. Method according to one of the preceding claims, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

22. Method according to one of the preceding claims, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

23. Method according to one of the preceding claims, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

24. Method according to one of the preceding claims, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

25. Method according to one of the preceding claims, where the coat protein is derived from polyomavirus.

26. Method according to one of the preceding claims, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

27. Method according to one of the preceding claims, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

28. Method according to one of the preceding claims, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

29. Method according to one of the preceding claims, where the cell is a vertebrate cell or a human cell.

30. Method according to one of the preceding claims, where at least two dsRNAs which differ from each other are introduced into the cell, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

31. Method according to one of the preceding claims, where one of the target genes is the PKR gene.

32. Medicament with at least one oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region which is complementary to the target gene,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

33. Medicament according to claim 32, where the dsRNA is enclosed by micellar structures, preferably by liposomes.

34. Medicament according to either of claims 32 or 33, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

35. Medicament according to one of claims 32 to 34, where the target gene can be expressed in eukaryotic cells.

36. Medicament according to one of claims 32 to 35, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

37. Medicament according to one of claims 32 to 36, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

38. Medicament according to one of claims 32 to 37, where the target gene is part of a virus or viroid.

39. Medicament according to claim 38, where the virus is a virus or viroid which is pathogenic for humans.

40. Medicament according to claim 38, where the virus or viroid is a virus or viroid which is pathogenic for animals.

41. Medicament according to one of claims 32 to 40, where segments of the dsRNA are in double-stranded form.

42. Medicament according to one of claims 32 to 40, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

43. Medicament according to one of claims 32 to 42, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

44. Medicament according to one of claims 32 to 43, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

45. Medicament according to one of claims 32 to 44, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

46. Medicament according to one of claims 32 to 45, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

47. Medicament according to one of claims 32 to 46, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

48. Medicament according to one of claims 32 to 47, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

49. Medicament according to one of claims 32 to 48, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

50. Medicament according to one of claims 32 to 49, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)-cystamine; 4-thiouracil; psoralene.

51. Medicament according to one of claims 32 to 50, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

52. Medicament according to one of claims 32 to 51, where the chemical linkage are [sic] triple-helix bonds provided at the ends of the double-stranded structure.

53. Medicament according to one of claims 32 to 52, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

54. Medicament according to one of claims 32 to 53, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

55. Medicament according to one of claims 32 to 54, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

56. Medicament according to one of claims 32 to 55, where the coat protein is derived from the polyomavirus.

57. Medicament according to one of claims 32 to 56, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

58. Medicament according to one of claims 32 to 57, where, when a capsid or capsid-type structure is formed from the coat protein, one side faces the interior of the capsid or capsid-type structure.

59. Medicament according to one of claims 32 to 58, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

60. Medicament according to one of claims 32 to 59, where the cell is a vertebrate cell or a human cell.

61. Medicament according to one of claims 32 to 60, where at least two dsRNAs which differ from each other are contained in the medicament, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

62. Medicament according to claim 61, where one of the target genes is the PKR gene.

63. Active ingredient with at least one oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region which is complementary to the target gene, and where the target gene is part of a phytopathogenic virus or viroid,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

64. Active ingredient according to claim 63, where the target gene can be expressed in eukaryotic cells.

65. Active ingredient according to claim 63 or 64, where segments of the dsRNA are in double-stranded form.

66. Active ingredient according to one of claims 63 to 65, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

67. Active ingredient according to one of claims 63 to 66, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

68. Active ingredient according to one of claims 63 to 67, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

69. Active ingredient according to one of claims 63 to 68, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

70. Active ingredient according to one of claims 63 to 69, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

71. Active ingredient according to one of claims 63 to 70, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

72. Active ingredient according to one of claims 63 to 71, where the chemical linkage is formed by azabenzene units inserted into the double-stranded structure.

73. Active ingredient according to one of claims 63 to 72, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

74. Active ingredient according to one of claims 63 to 73, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene.

75. Active ingredient according to one of claims 63 to 74, where the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded structure.

76. Active ingredient according to one of claims 63 to 75, where the chemical linkage are triple-helix bonds provided at the ends of the double-stranded structure.

77. Active ingredient according to one of claims 63 to 76, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

78. Active ingredient according to one of claims 63 to 77, where at least one nucleotides at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

79. Active ingredient according to one of claims 63 to 78, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

80. Active ingredient according to one of claims 63 to 79, where at least two dsRNAs which differ from each other are contained in the active ingredient, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

81. Use of an oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands or preparing a medicament or active ingredient for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region which is complementary to the target gene,
characterized in that
the complementary region has less than 25 successive nucleotide pairs.

82. Use according to claim 81, where the dsRNA is enclosed by micellar structures, preferably by liposomes.

83. Use according to either of claims 81 or 82, where the dsRNA is enclosed by natural viral capsids or by chemically or enzymatically produced artificial capsids or structures derived therefrom.

84. Use according to one of claims 81 to 83, where the target gene can be expressed in eukaryotic cells.

85. Use according to one of claims 81 to 84, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

86. Use according to one of claims 81 to 85, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

87. Use according to one of claims 81 to 86, where the target gene is part of a virus or viroid.

88. Use according to claim 87, where the virus is a virus or viroid which is pathogenic for humans.

89. Use according to claim 87, where the virus or viroid is a virus or viroid which is pathogenic for animals or phytopathogenic.

90. Use according to one of claims 81 to 89, where segments of the dsRNA are in double-stranded form.

91. Use according to one of claims 81 to 90, where the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

92. Use according to one of claims 81 to 91, where the cohesion of the double-stranded structure, which is caused by the complementary nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

93. Use according to one of claims 81 to 92, where the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

94. Use according to one of claims 81 to 93, where the chemical linkage is generated at at least one, preferably both, ends of the double-stranded structure.

95. Use according to one of claims 81 to 94, where the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

96. Use according to one of claims 81 to 95, where the chemical linkage is formed by purine analogs used in the double-stranded structure in place of purines.

97. Use according to one of claims 81 to 96, where the chemical linkage is formed by azabenzene units introduced into the double-stranded structure.

98. Use according to one of claims 81 to 97, where the chemical linkage is formed by branched nucleotide analogs used in the double-stranded structure in place of nucleotides.

99. Use according to one of claims 81 to 98, where at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloro-ethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)-cystamine; 4-thiouracil; psoralene.

100. Use according to one of claims 81 to 99, where the chemical linkage is formed by thiophosphoryl groups attached to the ends of the double-stranded structure.

101. Use according to one of claims 81 to 100, where the chemical linkage at the ends of the double-stranded structure is formed by triple-helix bonds.

102. Use according to one of claims 81 to 101, where at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the double-stranded structure is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

103. Use according to one of claims 81 to 102, where at least one nucleotide in at least one strand of the double-stranded structure is a locked nucleotide with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

104. Use according to one of claims 81 to 103, where the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

105. Use according to one of claims 81 to 104, where the coat protein is derived from polyomavirus.

106. Use according to one of claims 81 to 105, where the coat protein contains the polyomavirus virus protein 1 (VP1) and/or virus protein 2 (VP2).

107. Use according to one of claims 81 to 106, where, when a capsid or capsid-type structure is formed

from the coat protein, one side faces the interior of the capsid or capsid-type structure.

108. Use according to one of claims 81 to 107, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

109. Use according to one of claims 81 to 108, where the cell is a vertebrate cell or a human cell.

110. Use according to one of claims 81 to 109, where at least two dsRNAs which differ from each other are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

111. Use according to claim 110, where one of the target genes is the PKR gene.

112. Use according to one of claims 81 to 111, where the medicament is injectable into the bloodstream or into the interstitium of the organism to undergo therapy.

113. Use according to one of claims 81 to 112, where the dsRNA is taken up into bacteria or microorganisms.

114. Use of a vector for coding at least one oligoribonucleotide with double-stranded structure (dsRNA) formed by two separate RNA single strands for preparing a medicament or active ingredient for inhibiting the expression of a given target gene, where one strand of the dsRNA has a region which is complementary to the target gene, characterized in that

the complementary region has less than 25 successive nucleotide pairs.

115. Use according to claim 114, where the target gene can be expressed in eukaryotic cells.

116. Use according to claim 114 or 115, where the target gene is selected from the following group: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

117. Use according to one of claims 114 to 116, where the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

118. Use according to one of claims 114 to 117, where the target gene is part of a virus or viroid.

119. Use according to claim 118, where the virus is a virus or viroid which is pathogenic for humans.

120. Use according to claim 118, where the virus or viroid is a virus or viroid which is pathogenic for animals or phytopathogenic.

121. Use according to one of claims 114 to 120, where segments of the dsRNA are in double-stranded form.

122. Use according to one of claims 114 to 121, where one strand of the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

123. Use according to one of claims 114 to 122, where the cell is a vertebrate cell or a human cell.

124. Use according to one of claims 114 to 123, where at least two dsRNAs which differ from each other

are used, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

125. Use according to claim 125, where one of the target genes is the PKR gene.

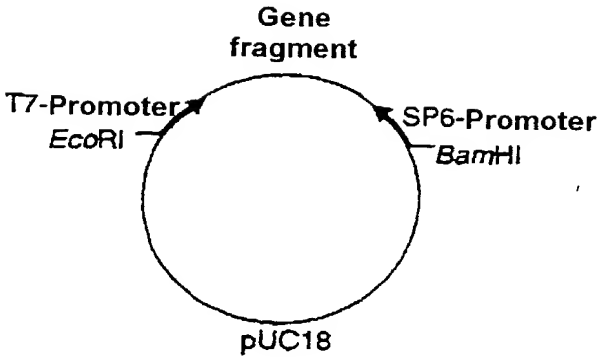

Fig. 1

Fig. 2

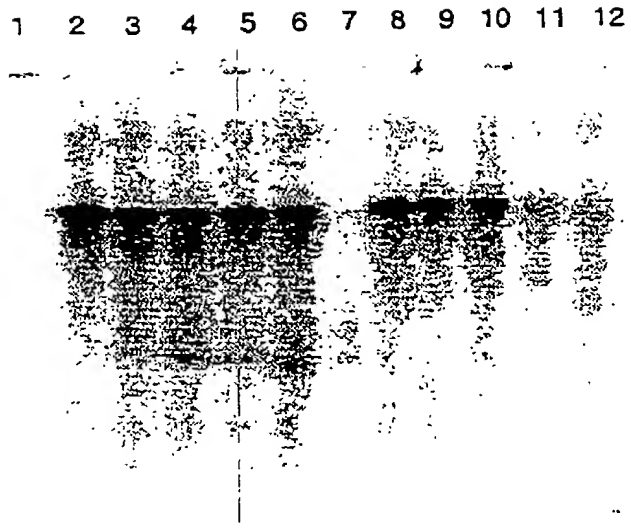

Fig. 3

Fig. 4 a

Fig. 4 b

Fig. 4 c

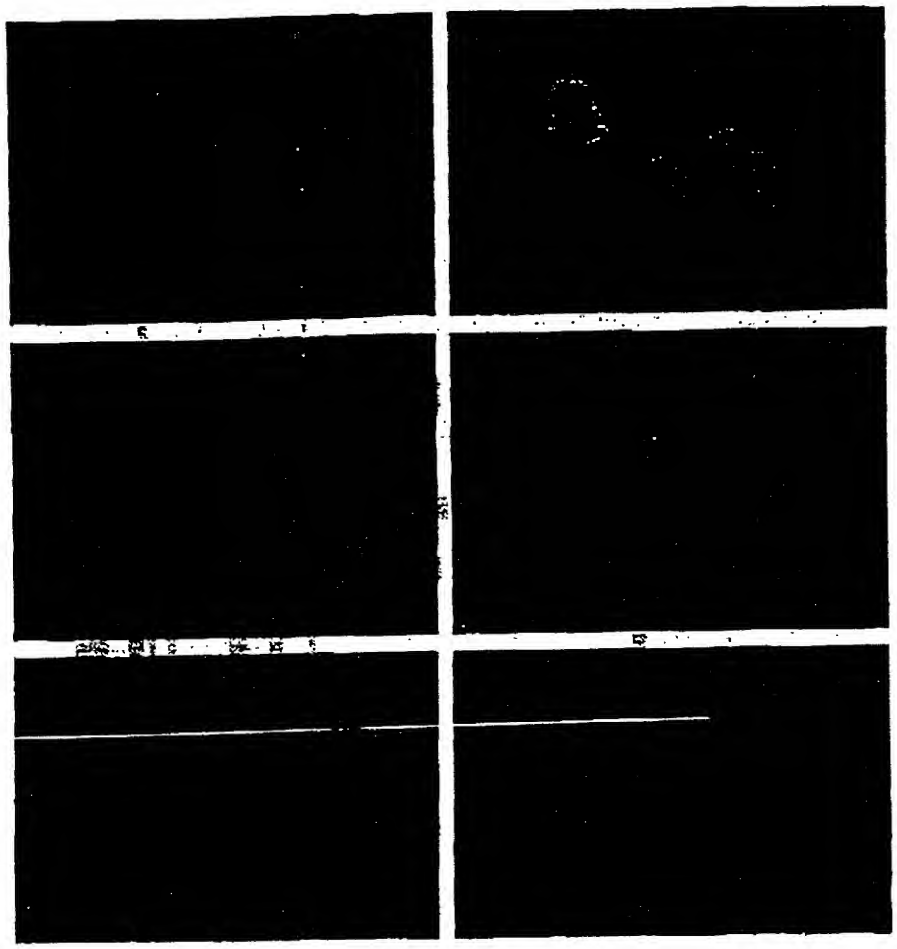

Fig. 4 d

Fig. 4 e

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DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION

[ ] Submitted with Initial Filing

[X] Submitted after Initial Filing
(Surcharge (37 CFR 1.16(e)) required)

Attorney Docket No.: 33796

Application Number: 09/889,802

First Named Inventor: Roland Kreutzer

Filing Date: July 20, 2001

Group Art Unit: _____

Examiner Name: _____

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD AND MEDICAMENT FOR INHIBITING THE EXPRESSION OF A DEFINED GENE

the specification of which (check only one item below)

[ ] is attached hereto,

OR

[X] was filed on July 20, 2001 as United States Application Number or PCT International Application Number 09/889,802 and was amended on July 20, 2001.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d), or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Country	Prior Foreign Application Number(s)	Foreign Filing Date (MM/DD/YYYY)	Priority Claimed?
Germany	199 03 713.2	January 30, 1999	Yes
Germany	199 56 568.6	November 24, 1999	Yes
PCT	PCT/DE00/00244	January 29, 2000	Yes

I hereby claim the benefit under 35 U.S.C. 120, of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
PCT/DE00/00244	January 29, 2000	

As a named inventor, I hereby appoint all practitioners at Customer No. 000116 as my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all correspondence to Customer Number 000116.

Please direct all correspondence and inquiries to John P. Murtaugh at (216) 579-1700.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature: X _____

Date: X 08/31/2001

Citizenship: Germany

Residence (City, Country): Bayreuth, Germany DEX

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D-95447 Bayreuth, Germany

Sequence Listing

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Limmer Dr., Stephan

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Limmer Dr., Stephan

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gcuguaggca uaggcuuggu uaugccggua cugccgggcc ucuugcggga uaucguccau 180
uccgacagca ucgccaguca cuauggcgug cugcuagcgc uauaugcguu gaugcaauuu 240
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